METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF OFLOXACCIN AND SATRANIDAZOLE BY USING RP-HPLC

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ABSTRACT

A new simple, accurate, rapid and precise gradient High Performance Liquid Chromatographic (HPLC) method was developed and validated for simultaneous estimation of Ofloxacin and satranidazole in combined dosage form. The method employs waters HPLC system on Hypersil BDS C₁₈ Column (250 mm X 4.6 mm, 5 μ), with a flow rate of 1.0 ml / min with a load of 10 μ l. Water + OPA (Ortho Phosphoric Acid) having pH 5.0 and acetonitrile was used as a mobile phase in the composition of 60:40 % v/v. The detection was carried out by PDA detector at 295 nm. Linearity ranges for Ofloxacin and satranidazole were 100 – 300 μ g / ml for Ofloxacin, 150 – 450 μ g / ml for satranidazole respectively. Retention time of Ofloxacin and Satranidazole were found to be 2.881 minutes, 4.042 minutes respectively. Percent recovery study values of Ofloxacin and Satranidazole were found to be 99.003 – 100.5 % and 98.6 – 100.4 % respectively. This newly developed method was successfully utilized for the quantitative estimation of Ofloxacin and Satranidazole in combined pharmaceutical dosage forms. This method was validated as per ICH guidelines.

Keywords: Ofloxacin, Satranidazole RP – HPLC, validation, simultaneous estimation.

INTRODUCTION

Ofloxacin is chemically 9-fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-Pyrido [1, 2, 3-de]-1, 4-benzoxazine-6-carboxylic acid] (Figure 1). Ofloxacin acts on DNA gyrase and topoisomerase IV, enzymes which, like human topoisomerase, prevents the excessive super coiling of DNA during replication or transcription. By inhibiting their function, the drug thereby inhibits normal cell division. Satranidazole is chemically 1-(1-Methyl-5-nitro-1H-imidazol-2-yl)-3-(methylsulfonyl)-2-imidazolidinone (Figure 2), is a nitro imidazole's which have broad spectrum bactericidal activity against Protozoa and some anaerobic bacteria. Its selective toxicity to anaerobic microbes involves

- Drug enters the cell by diffusion,
- Nitro group of drug is reduced by redox proteins present only in anaerobic organisms to reactive nitro radical which exerts cytotoxic action by damaging DNA and other critical biomolecules.
- DNA helix destabilization and strand breakage has been observed.

Literature survey reveals the availability of several UV spectroscopy^{1, 2} and RP – HPLC³⁻⁷ methods for estimation of Ofloxacin and Satranidazole. The current available methods are developed for very short runtime and the resolution was also less. The objective of the present work was to develop and validate new analytical method which provides improved analytical identification or detection limits, greater resolution, accuracy and precision.

MATERIALS AND METHODS

Instrumentation

High performance liquid chromatography (waters) with quaternary pump, PDA detector and with Hypersil BDS C₁₈ Column (250 mm X 4.6 mm, 5 μ) was used. The detection of drug was carried by UV detector data processing by Empower – 2 software, weighing balance (Mettler Toledo), sonicator (Elma S 300 H), pH meter (Inolab WTW series).

Reagents and Chemicals

Methanol (HPLC grade), water (HPLC), Potassium dihydrogen O-phosphate (GR Merck Chemicals), O-Phosphoric Acid (AR Rankem), Acetonitrile (GR Merck Chemicals) and pure drug samples of Ofloxacin and Satranidazole are gift samples from Rainbow pharma training lab, Hyderabad, India.

Chromatographic Condition

The analysis was carried out by HPLC using Water + OPA (Ortho Phosphoric Acid) having pH 5.0 as buffer and acetonitrile was used as a diluent in the composition of 60:40 % v/v and this was used as a mobile phase and Hypersil BDS C₁₈ Column (250 mm X 4.6 mm, 5 μ) as a stationary phase at a flow rate of 1.0 ml / min, in isocratic mode. With the above mobile phase good resolution between Ofloxacin and Satranidazole was achieved with a run time of 20 minutes. The injection volume was 10 μ l and the detection was performed at 295 nm. (Figure 5)

Solution Preparation

Preparation of Buffer solution (pH 5.0)

Accurately measured quantity of 1000 ml of HPLC Grade water was taken and its P^H was adjusted to 5.0 with 5 % (v/v) Ortho-phosphoric acid solution and degassed.

Preparation of Mobile Phase

Buffer and Acetonitrile were mixed in the ratio of 60:40 v/v respectively and degassed.

Preparation of Diluent

Mobile phase was used as the diluent.

Preparation of Standard Solution

Required quantity was weighed accurately and about 30 mg of Satranidazole working standard, 20.2 mg of Ofloxacin working standard was transferred into a 100 ml volumetric flask and dissolved in 20 ml diluent by sonicating for 10 minutes and was made up to the volume with diluent. The

above solution was filtered through 0.45 µm PVDF membrane filter.

Calibration Curves

Linearity experiments were performed by giving five replicates for both the components and the response was found to be linear in the range of 100 to 300 ppm for Ofloxacin and 150 to 450 ppm for Satranidazole. The linearity of Ofloxacin and satranidazole was plotted by a graph between response factor and concentration. (Figure 3,4).

Analysis of Tablet Formulation

A quantity of the mixed contents was weighed accurately that is 20 tablets equivalent to about 200 mg of Ofloxacin and transferred into a 100 ml volumetric flask. 20 ml of diluent was added and sonicated for about 10 minutes and diluted to the volume with diluent. 5 ml of the above solution was further diluted to 50 ml with diluent and mixed. The above solution was filtered through 0.45 µm PVDF membrane filter.

Procedure

The column was equilibrated for not less than 30 minutes with initial gradient at a flow rate of 1.0 ml / min. 10 µl of blank solution (Diluent), standard solution (5 injections) and sample solution were injected separately into the chromatographic system. The chromatograms were recorded and the peak responses were measured.

Validation of HPLC Method

Specificity

The specificity of the RP – HPLC method was determined by comparison of the chromatogram of mixed standard and sample solution which were injected separately and the parameters like retention time, resolution and tailing factor were calculated. Good correlation was found between the results of mixed standards and sample solutions.

Precision

Precision of proposed HPLC method was found to be 0.309 (% RSD) for Ofloxacin and 0.168 (% RSD) for

Satranidazole that indicates good precision of the sample analyzed. Table 3, 4 shows the data.

Accuracy (Recovery Studies)

Accuracy of method was calculated by recovery studies (n = 6) at six levels. Standard drug solution containing drugs in the range for Ofloxacin and Satranidazole of concentration was added to previous analyzed test solution. Amount of drug recovered at each level (n = 6) was determined. Percent recovery at each level was calculated. Table 5, 6 shows data from the recovery study for Ofloxacin, satranidazole were 99.003 – 100.5 % for Ofloxacin, 98.6 – 100.4 % for satranidazole, respectively. High percentage recovery showed that the method was free from interference of excipients used in formulation.

Robustness

Robustness of the method was checked by changing flow rate, temperature, and standard was able to give system suitability parameters within limit, which indicates that the method is robust results are shown in Table 7.

System Suitability Parameters

The system suitability parameters were evaluated. The % RSD for five replicate injections of Ofloxacin and Satranidazole was 0.309 and 0.168 and found to be within the limits. The tailing factors for Ofloxacin and satranidazole peaks were 1.6 and 1.16 respectively. The theoretical plates for Ofloxacin and satranidazole are 41549 and 6809 were found to be within the limits. The system suitability parameters were evaluated and found to be within the limits. Results are shown in Table 8.

RESULTS AND DISCUSSION

The proposed method was a RP – HPLC procedure which employed a Hypersil BDS C₁₈ (250 mm X 4.6 mm, 5 µ) column. Water + OPA (pH 5.0) and acetonitrile in the composition of 60:40 % v/v was used as the mobile phase. The drug solutions were scanned in the range of 200 to 400 nm on a UV – Visible spectrophotometer for selection of sampling wavelength. After recording the spectra of the two drugs, 295 nm was selected as a suitable wavelength for estimation.

Table 1: Linearity of Detection Response for Ofloxacin

Ofloxacin	
Conc. (mcg)	Area
100	197812
150	286544
200	385706
250	475264
300	581348

Table 3: Method Precision for Ofloxacin

Parameters	Rt (min)	Area
Ofloxacin	2.891	388313
	2.892	390522
	2.892	389639
	2.891	391354
	2.892	388315
	2.891	389832
Mean	2.892	389662
SD	0.00054	1206
% RSD	0.309	0.3

Table 2: Linearity of Detection Response for Satranidazole

Satranidazole	
Conc. (mcg)	Area
150	261480
225	379584
300	514161
375	636879
450	775303

Table 4: Method Precision for Satranidazole

Parameters	Rt (min)	Area
Satranidazole	3.839	520209
	3.841	520637
	3.841	522053
	3.841	519693
	3.841	522381
	3.840	519934
Mean	3.834	520818
SD	0.0161	1133.04
%RSD	0.168	0.2

Table 5: Accuracy Results of Ofloxacin

Drug	Sample	Sample weight (mcg)	Amount Added (mcg / ml)	Amount Estimated (mcg / ml)	% Recovery	% mean
Ofloxacin	50 % - 01	320.0	100.6	102.9	102	102
	50 % - 02	320.0	100.6	103.8	102	
	50 % - 03	320.0	100.6	102.2	102	
	50 % - 04	320.0	100.6	102.8	102	
	50 % - 05	320.0	100.6	103.3	103	
	50 % - 06	320.0	100.6	100.63	100	
	100 % - 01	636.0	200.0	195.41	98	98
	100 % - 02	636.0	200.0	198.32	99	
	100 % - 03	636.0	200.0	195.17	98	
	150 % - 01	953.0	299.6	297.19	99	100
	150 % - 02	953.0	299.6	298.9	100	
	150 % - 03	953.0	299.6	299.6	100	
	150 % - 04	953.0	299.6	298.5	100	
	150 % - 05	953.0	299.6	302.12	101	
	150 % - 06	953.0	299.6	301.59	101	

Table 6: Accuracy Results of Satranidazole

Drug	Sample	Sample weight (mcg)	Amount Added (mcg / ml)	Amount Estimated (mcg / ml)	% Recovery	% mean
Satranidazole	50 % - 01	320.0	150.9	152.9	101	101
	50 % - 02	320.0	150.9	154.3	102	
	50 % - 03	320.0	150.9	154.5	102	
	50 % - 04	320.0	150.9	153.5	102	
	50 % - 05	320.0	150.9	153.7	103	
	50 % - 06	320.0	150.9	147.0	97	
	100 % - 01	636.0	300.0	293.2	98	98
	100 % - 02	636.0	300.0	293.0	98	
	100 % - 03	636.0	300.0	291.7	97	
	150 % - 01	953.0	449.5	445.1	99	100
	150 % - 02	953.0	449.5	444.2	99	
	150 % - 03	953.0	449.5	447.6	100	
	150 % - 04	953.0	449.5	445.6	99	
	150 % - 05	953.0	449.5	460.7	102	
	150 % - 06	953.0	449.5	451.4	100	

Table 7: Robustness for Ofloxacin and Satranidazole

	Flow rate	Ofloxacin		Satranidazole	
		Area	R _t	Area	R _t
	0.9 ml	394500	3.5	540165	4.7
	1.1 ml	387868	2.4	534568	3.2
	% RSD	1.19		0.736	
Temperature	Ofloxacin		Satranidazole		
	Area	R _t	Area	R _t	
35°C	389921	2.8	516701	3.8	
45°C	388412	2.8	516711	3.8	
% RSD	0.274		0.01		

Table 8: System Suitability

Parameters	R _t	Resolution	Theoretical plates	Tailing factor
Ofloxacin	2.890	--	5705	0.98
	2.890	--	5684	1.02
	2.891	--	5624	1.06
	2.892	--	5584	1.12
	2.891	--	5494	1.15
Satranidazole	3.838	5.24	6982	0.93
	3.838	5.31	6914	0.96
	3.838	5.45	6863	0.99
	3.839	5.52	6798	1.05
	3.839	5.60	6732	1.18

Table 9: Results of LOD LOQ

Sample	LOD Value (µg / ml)	LOQ Value (µg / ml)
Ofloxacin	1.37	7.0
Satranidazole	2.0	10.6

Figure 1: Ofloxacin structural Formula

Figure 2: Satranidazole structural Formula

Figure 3: Linearity Curve for Ofloxacin

Figure 4: Linearity Curve for Satranidazole

Figure 5: Typical Chromatogram of Ofloxacin and Satranidazole

Name	RT	Area	% Area	Height	Resolution	s/n	USP Tailing	USP Plate Count
Ofloxacin	2.876	463601	50.78	96190	7	122.81	1.233	8862
Satranidazole	3.838	449289	49.22	77557		99.02	1.095	10917

The best resolution between Ofloxacin and Satranidazole was achieved with in 6 minutes of run time, with flow rate of 1.0 ml / min. The linearity response of the HPLC system for Ofloxacin was found to be in the range of 100 to 300 ppm and for Satranidazole in the range of 150 to 450 ppm. Accuracy of the method was checked by adding standard drug solution containing drugs within the range. The recovery study for Ofloxacin was between 99.003 – 100.5 % and 98.6 – 100.4 % for Satranidazole. Precision of the method was found to be 0.309 (% RSD) for Ofloxacin and 0.168 (% RSD) for Satranidazole that indicates good precision of the sample analyzed. When changing the flow rate and temperature of the method, the system suitability parameters remained within limits, so that the method was proved to be robust. The LOD, LOQ values of Ofloxacin were 1.37, 7.0 and Satranidazole 2.0, 10.6 respectively (Table 9). The other validation parameters, sensitivity and selectivity of the proposed method were found be within limits as the number of theoretical plates, tailing factor, resolution, separation factor, results of analysis and statistical parameters.

CONCLUSION

The present work describes simple, economical and non-interfering simultaneous method of estimation of Ofloxacin and Satranidazole by using RP – HPLC. The method was found to be economic, simple, precise, accurate and reproducible during analysis of drug formulations containing the two drugs.

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